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14. ABSTRACT

Autism spectrum disorder (ASD) is a polygenic signaling disorder that may result, in part, from an imbalance in excitatory and inhibitory neurotransmission at the network level which strongly suggests that inhibitory neurotransmission plays a key role; perhaps as a result of deficits in γ-aminobutyric acid-A receptor ($GABA_AR$) mediated signaling. Therefore $GABA_ARs$ may be a relevant therapeutic target for blocking or reversing the symptoms of ASD. Nicotinic cholinergic activity may supplement/enhance GABA efficacy on cognitive function. Significant cognitive deficits are associated with ASD and extensive preclinical studies/clinical proof-of-concept trials support a crucial role for α7 nicotinic acetylcholine (nACh) receptors learning and memory. Our studies test the hypothesis that the simultaneous and selective positive allosteric modulators (PAMs) of $GABA_A$ & α7 nACh receptors will alleviate the core deficits and significant comorbidities of ASD. Our major findings are 1) selective PAMs of β_1-subunit containing $GABA_A$ receptor subtypes, by the test compound 2-261, positively impacts aberrant behaviors reflected in sociability (social interaction) but has no effect on repetitive behavior (stereotypy) in the BTBR mouse model of ASD and 2) the selective α7 nACh receptor PAM, AVL-3288, improves both social interaction and stereotypy in BTBR mice. Collectively, our observations plus existing data provide robust support, in part, for our working hypothesis and the unanticipated finding that a α7 nACh receptor PAM provides a strong rationale for the clinical testing of AVL-3288 in ASD.

15. SUBJECT TERMS

Gamma-amino-butyric acid, inhibitory, excitatory, neurotransmission, autism, social interaction, stereotype behavior, autism spectrum disorder, nicotinic, receptor, mouse

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Table of Contents

1. **INTRODUCTION:** Autism spectrum disorder (ASD) is a disease of development characterized by three core behavioral symptoms including difficulties in social interaction, verbal and nonverbal communication and repetitive/stereotypical behaviors. The Department of Health & Human Services states that the increasing prevalence of ASDs, currently estimated at 1 in 88 children, is a national health emergency. Yet there are no drugs for the treatment of these core deficits or associated neurological/medical symptoms such as epilepsy and anxiety. Consequently, ASD is a dire unmet medical need. The greatest challenge is to find a drug with a broad range of activity that will treat both the core symptoms and associated difficulties (i.e., epilepsy, anxiety, disrupted learning and memory). The objective of our project is to fulfill this profound need for drugs that can do exactly that by studying a new class of compounds that will simultaneously enhance the function of two neurotransmitters in the brain known as γ-aminobutyric acid (GABA) and acetylcholine (ACh). GABA acting through $GABA_A$ receptors ($GABA_A$Rs) is responsible for reducing the activity of nerve cells in the brain that may be over-stimulated in ASD and thus contributes to the three core symptoms and the anxiety and epilepsy that sometimes occur in ASD. ACh acting through α7 nicotinic receptors (α7 nAChRs) control the activity of nerve cells in the brain that may be under-stimulated in ASD which may underlie the difficulties in learning and memory observed in ASD. This approach may provide the basis for the design of an innovative series of drugs acting simultaneously at both receptors and will thus represent the first attempt at treating the core and significant comorbidities of ASD with a single drug.

2. **KEYWORDS:**

Alpha7 nicotinic receptors (α7 nAChRs), allosteric modulator, anxiety, autism spectrum disorder, brain, childhood disorders, comorbidity, epilepsy, excitatory, γ-aminobutyric acid (GABA), GABA type A receptor ($GABA_A$R), inhibitory neurotransmitter, learning, memory, mouse, self-grooming, sociability, social interaction

3. OVERALL PROJECT SUMMARY:

The project summary covers the entire duration of the project and follows exactly the activities described under each task (in **bold blue font**) in the approved Statement of Work (SOW). The narratives of the activities related to each task appear in *italics*.

Task 1: Seek animal use approval through local Institutional Animal Care and Use Committee (IACUC) and Department of Defense (DOD) Animal Care and Use Review Office (ACURO).

Task 1 was accomplished in the middle of the month of October, 2013. All animal protocols for the proposed studies received approval from the IACUC at UC Irvine and the ACURO.

The initiation of a breeding colony to provide BTBR mice for the studies was started in November, 2013. Sufficient numbers of mice for studies specified in task 2 occurred in February, 2014 and took about one month longer than predicted in the approved SOW.

Task 2: (Specific Aim 1): Do single site positive allosteric modulators (PAMs) of α7 nACh or $GABA_A$ subtype (i.e., $\beta_{2/3}$-subunit containing) receptors correct any of the core ASD-related symptoms and comorbidities in the mouse models?

Task 2a: Synthesis of 2-261, AVL-3288 & GRN-529.

This task was accomplished in December, 2013. The task was accomplished one month later than predicted in the approved SOW because of the need to synthesize some of the starting materials that were commercially unavailable for the synthesis of the compounds of interest.

Task 2b: Training on behavioral paradigms using BTBR mice.

Training was completed in December, 2013 utilizing the BTBR mice from the breeding colony. This task was completed one month later than anticipated due to the one month delay in breeding BTBR mice in sufficient numbers to satisfy demand as described in task 1.

Task 2c: Pharmacokinetics – confirm brain penetrability in BTBR mice.

The brain penetrability of the test compounds 2-261 and AVL-3288 were determined in BTBR mice and found to be brain penetrant and consistent with observations in other strains of mice that we have tested in the past. The plasma and brain levels of 2-261 following a 10 mg/kg i.p. dose is shown in table 1. The brain levels of 2-261 achieved at 10 mg/kg i.p. are well in excess of that required to activate the receptor (when based on the EC_{50} of 2-261 at the $GABA_A$ receptor i.e., saturating concentrations) as measured electrophysiologically. Comparable brain levels of AVL-3288 were also achieved after a 10 mg/kg i.p. dose (data not shown). This task was completed in February, 2014, about one month later than predicted in the SOW due to a one month delay in generating sufficient numbers of BTBR mice in the breeding colony.

Table 1. Plasma and brain levels of 2-261 at 30 and 60 minutes after a 10 mg/kg i.p. dose

Time (minutes) after i.p. administration	Plasma levels (μM)±SEM	Brain levels (μM)±SEM
30	1.5 ± 0.3	4.3 ± 1.0
60	0.6 ± 0.1	2.3 ± 0.4

SEM=standard error of the mean

Task 2d: Sociability – phenotype confirmation (BTBR strain).

The behavioral phenotype of the BTBR strain of mice was confirmed and found to be consistent with that reported in the literature (Silverman et al., 2012). The phenotype is shown in the right set of bars in figure 1 where BTBR mice spend significantly more time with the novel object over the novel mouse (DBA/2) in this sociability paradigm. As found in published reports from other groups, we have also observed no statistically significant differences in time spent between the novel object and mouse but we have never observed a significantly greater time spent with the novel mouse over the novel object. These studies were completed in March, 2014, one month later than anticipated due to the delay in generating sufficient numbers of BTBR mice in the breeding colony.

Task 2e: Sociability – phenotype confirmation (C57BL/6J and BTBR strains).

The behavioral phenotype of the C57BL/6J strain of mice was confirmed and consistent with that reported in the literature (Silverman et al., 2012). The phenotype is shown in figure 1 where C57BL/6J mice spend significantly more time with the novel mouse than the novel object in this sociability paradigm and the BTBR mice spend significantly more time with the novel object than the novel mouse. We also observed sessions in both strains where there were no differences in sniffing times between the novel object versus novel mouse. However we have never observed sessions where C57BL/6J mice show increased interaction with the novel object over the novel mouse which is also consistent with literature reports.

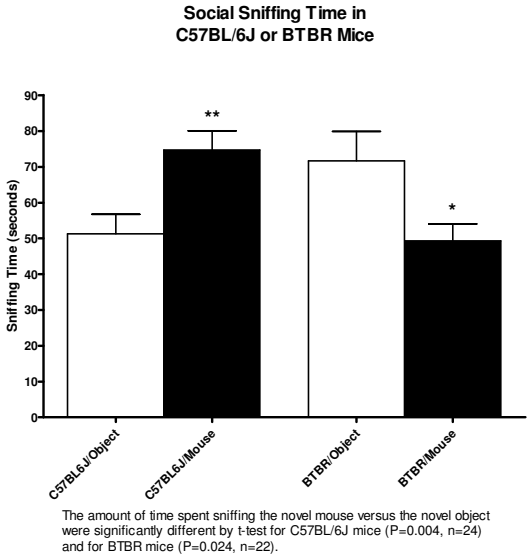

Figure 1: The sociability of C57BL/6J or BTBR mice was recorded as the time in seconds spent sniffing the novel mouse (filled bars) versus the novel object (open bars).

Task 2f & 2g: Sociability – drug testing (BTBR strain).

Three drugs, the positive control GRN-529, 2-261, AVL-3288 and vehicle were tested in BTBR mice to determine their effects on sociability as measured by the difference in time they spend with a novel object versus a novel mouse (DBA/2) in the sociability paradigm. Figure 2 shows the effect of the positive control GRN-529 (a negative allosteric modulator of mGluR5 at 3 mg/kg i.p.) on sociability in BTBR mice. Consistent with the effect of this compound reported in the literature (Silverman et al., 2012), it significantly altered the preference of the BTBR mice by reducing the time spent with the novel object while increasing time spent with the novel mouse. When the $GABA_A$ receptor subtype selective modulator 2-261 was tested in this paradigm, it showed an effect similar to GRN-529 where there was an increase in time spent with the novel mouse relative to the time spent with the novel object at doses of 0.3, 1 and 3 mg/kg i.p. (Figure 3, left panel). When the $\alpha 7$ nAChR modulator AVL-3288 was tested in this paradigm, it showed an effect similar to GRN-529 where there was an

increase in time spent with the novel mouse relative to the time spent with the novel object at the dose of 3 mg/kg i.p. (Figure 3, right panel).

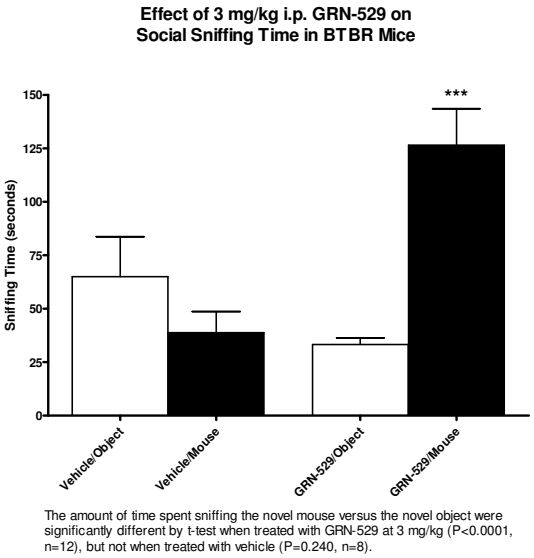

Figure 2: The effect of GRN-529 (3 mg/kg i.p.) on sociability in BTBR mice was recorded as the time in seconds (sec) spent sniffing the novel mouse (filled bars) versus the novel object (open bars) starting 30 minutes after vehicle or drug administration.

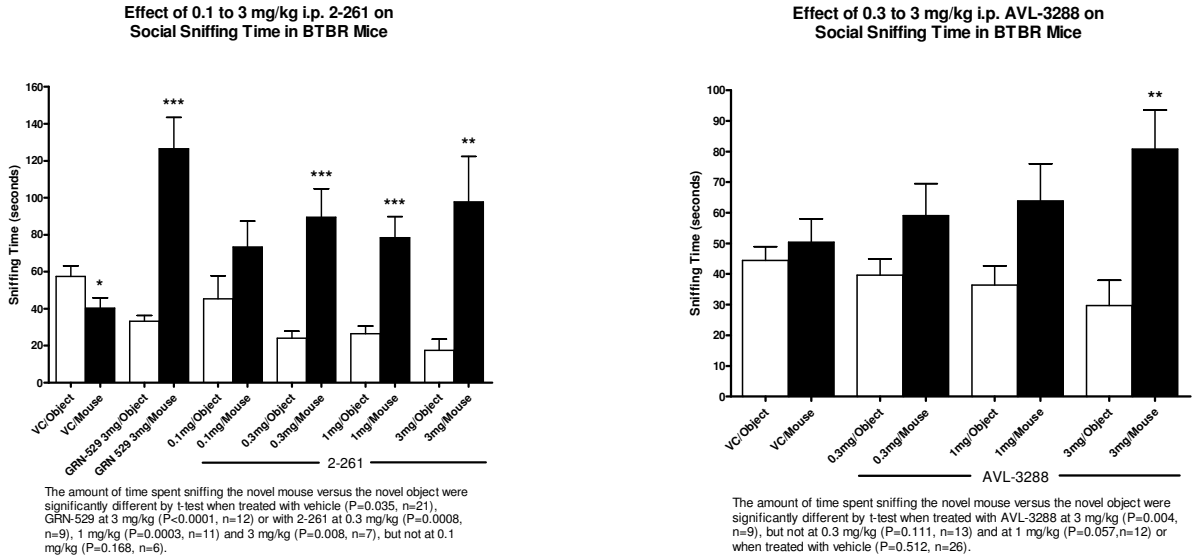

Figure 3: The effect of 2-261 (left panel, 0.1 to 3 mg/kg i.p.) or AVL-3288 (right panel, 0.3 to 3 mg/kg i.p.) on sociability in BTBR mice was recorded as the time in seconds (sec) spent sniffing the novel mouse (filled bars) versus the novel object (open bars) starting 30 minutes after vehicle or drug administration.

Task 2h & 2i: Sociability – drug effect specificity (C57BL/6J strain)

The absence of specificity of the effect of 2-261 at 1 mg/kg i.p. is shown in figure 4 where the drug had an effect on sociability on C57BL/6J mice. Thus both BTBR and C57BL/6J mice respond to the effect of 2-261 on sociability. The data suggest that the effect 2-261 on social interaction is not specific to the strain of mice.

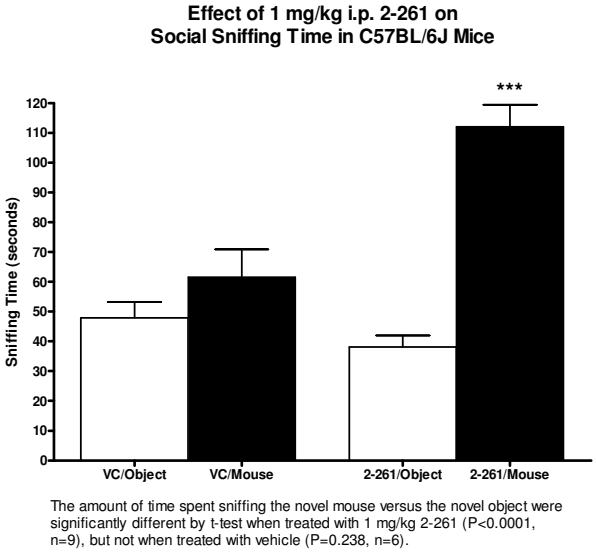

Figure 4: The effect of 2-261 (1 mg/kg i.p.) on sociability in C57BL/6J mice was recorded as the time in seconds (sec) spent sniffing the novel mouse (filled bars) versus the novel object (open bars) starting 30 minutes after vehicle or drug administration.

The DBA/2J mice appear to be effective as the novel mice in these social interaction studies where the same DBA/2J mouse is used for every 3 BTBR mice tested for a ratio of 1 DBA/2J to 3 BTBR mice. Initial testing was done with a ratio of 1:1. Subsequent testing with a ratio of 1:3 showed that no differences were observed in any measure when compared to a ratio of 1:1. This observation allowed us to use fewer novel mice per study.

Task 2j & 2k: Self-grooming– phenotype confirmation (BTBR versus C57BL/6J strains)

The behavioral phenotype of the BTBR strain of mice was confirmed and consistent with that reported in the literature (Silverman et al., 2012). The phenotype is shown in figure 5 where BTBR mice spend significantly more time self-grooming than the C57BL/6J mice.

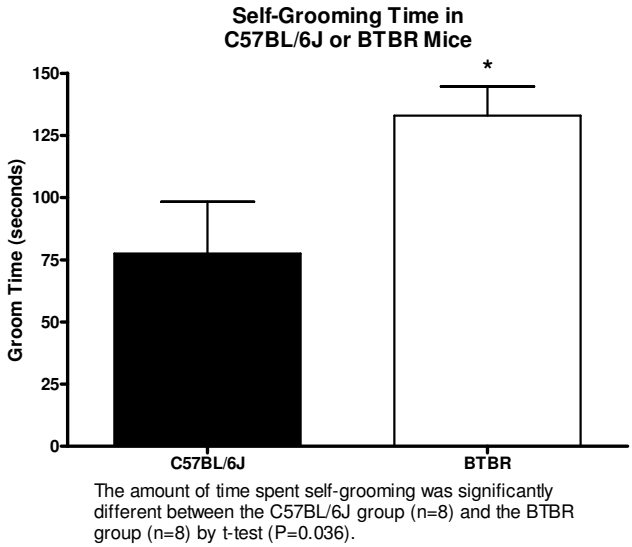

Figure 5: The self-grooming behavioral phenotype of C57BL/6J (open bar) versus BTBR (filled bar) strains of mice. Self-grooming behavior was measured during a 10 minute observation period.

Task 2l: Self-grooming – drug testing (BTBR strain)

Three drugs, the positive control GRN-529, 2-261, AVL-3288 and vehicle were tested in BTBR mice to determine their effects on repetitive behavior as measured by the change in the amount of time spent self-grooming. Figure 6 shows the effect of the positive control GRN-529 (3 mg/kg i.p.) on self-grooming in BTBR mice. Consistent with the effect of this compound reported in the literature (Silverman et al., 2012), it significantly reduced the amount of time spent self-grooming in BTBR mice. When the $GABA_A$ receptor subtype selective modulator 2-261 was tested in this paradigm, it showed no effect at doses of 0.3, 1, 3 or 10 mg/kg i.p. (Figure 7, left panel). When the α7 nAChR modulator AVL-3288 was tested in the same paradigm, it showed an effect similar to GRN-529 where there was an increase in time spent with the novel mouse relative to the time spent with the novel object at doses of 3 and 10 mg/kg i.p. (Figure 7, right panel).

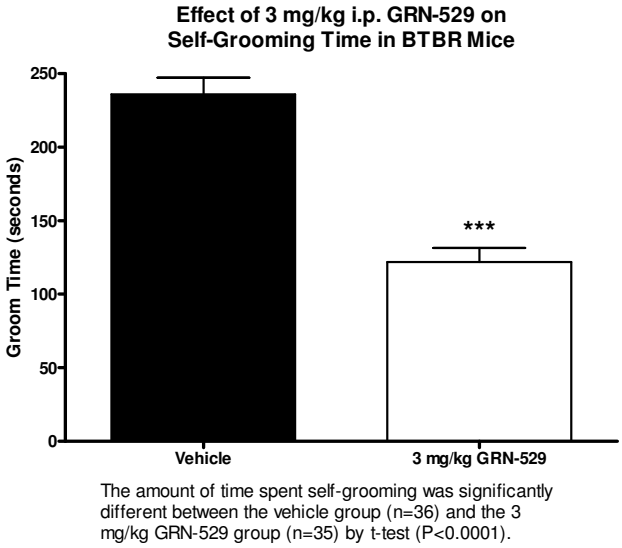

Figure 6: The effect of GRN-529 (3 mg/kg i.p.) on self-grooming behavior in BTBR mice. Self-grooming behavior was measured 30 minutes after vehicle or drug administration and the amount of time spent grooming was recorded during a 10 minute observation period.

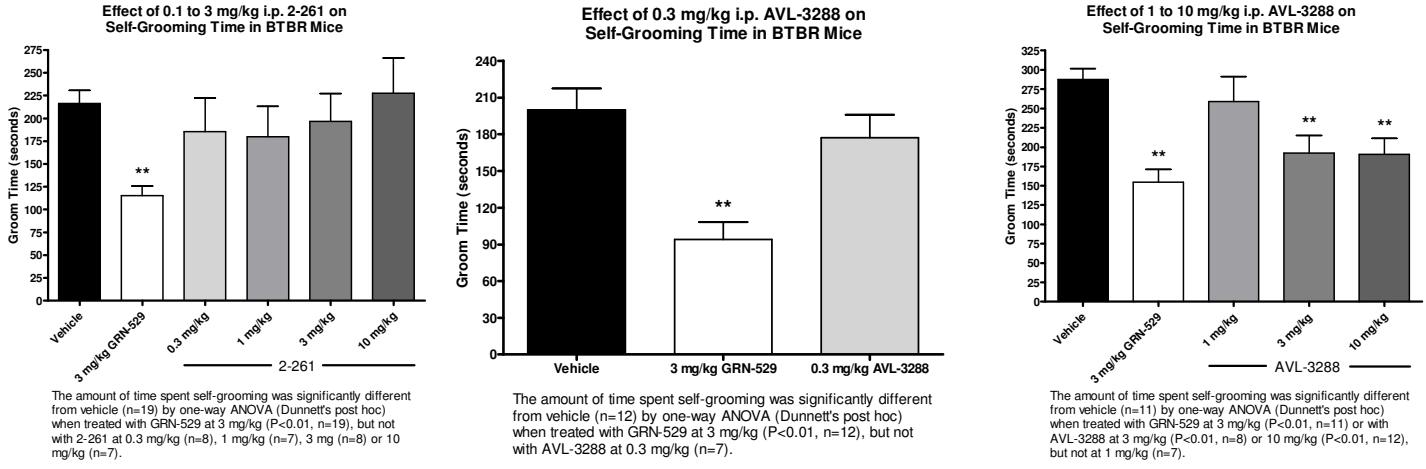

Figure 7: The effect of GRN-529 (3 mg/kg i.p.), 2-261 (left panel, 0.3 to 10 mg/kg i.p.) or AVL-3288 (center and right panels, 0.3 to 10 mg/kg i.p.) on self-grooming behavior in BTBR mice. Self-grooming behavior was measured 30 minutes after vehicle or drug administration and the amount of time spent grooming was recorded during a 10 minute observation period.

Task 2m: Self-grooming – drug effect specificity (C57 strain)

Drug specificity of AVL-3288 in self-grooming is shown by the lack of effect of a 3 mg/kg i.p. dose on this behavior in C57BL/6J mice whereas this dose was active in the BTBR mice (Figure 8). 2-261 was not tested in C57BL/6J mice because it was inactive in BTBR mice at all doses tested (see Figure 7, left panel).

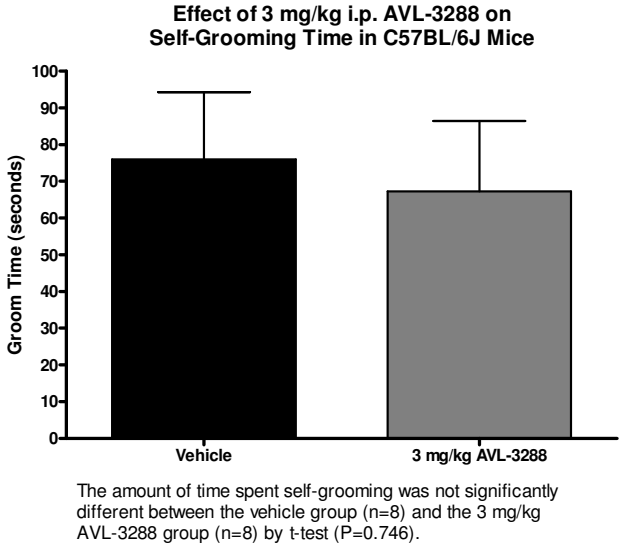

Figure 8: The effect of AVL-3288 (3 mg/kg i.p.) on self-grooming behavior in C57BL/6J mice. Self-grooming behavior was measured 30 minutes after vehicle or drug administration and the amount of time spent grooming was recorded during a 10 minute observation period.

Task 2n & 2o: 5-CSRTT – phenotype differentiation (BTBR and C57 strains)

The 5-choice serial reaction time task paradigm tests sustained attentional processes by giving the animals repeated trials where they are required to respond within a short amount of time to different stimuli that require specific responses. The animals are trained to criteria with longer stimulus durations and an increase in the amount of time to respond. The duration of the stimulus and the amount of time allowed for responding is then serially reduced to increase the attentional load. While BTBR mice demonstrated slower learning compared to C57BL/6J mice in the 5-CSRTT paradigm, the differences in accuracy once the animals reached criteria were not revealed until very short stimulus durations were used and even then they were marginal (McTighe et al., 2013).

We decided that it would be difficult to detect a treatment effect within that margin so we used the contextual rule switching paradigm that we originally proposed in the grant as alternative method to test executive function. It has been published that BTBR mice perform more poorly in rule switching paradigms (Rutz and Rothblat, 2012). We had some difficulty replicating these published results mainly due to both the BTBR and C57BL/6J mice learning extremely poorly in our setup. This resulted in an increase in the anticipated time (over 3 months longer than anticipated) required to acquire the data in these studies.

Task 2p: 5-CSRTT – drug testing (BTBR strain)

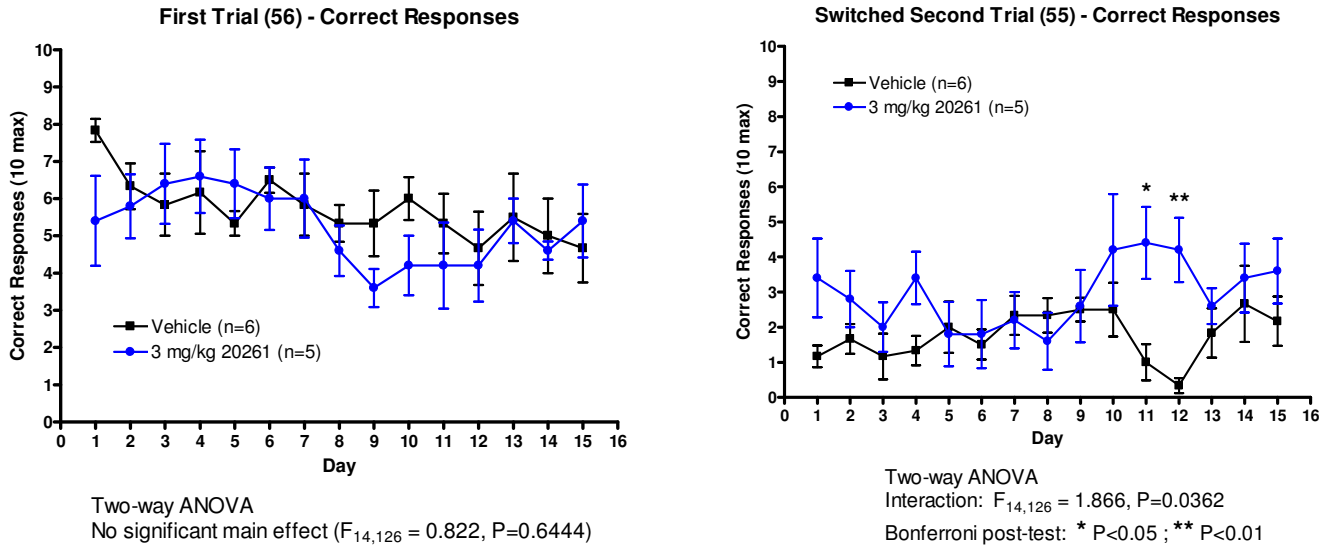

Figure 9: The effect of 2-261 (3 mg/kg i.p.) on contextual rule switching in BTBR mice. The ability for the animals to switch between two familiar and distinct rule sets in consecutive 10 trial sessions was measured after training to criteria at both rule sets individually. The first rule set required the animals to respond at the unlit nose-poke in response to one light stimulus pattern. The second rule set required the animals to respond at the lit nose-poke in response to a different light stimulus pattern.

We were able to get some data from the limited number of BTBR mice that were able to reach criteria. The data demonstrates that 2-261 does not have a significant effect on performance in the first familiar rule set (Figure 9, left panel), but when the animals are switched to the second familiar rule set there may be a limited increase on performance (Figure 9, right panel). This suggests that 2-261 may help these animals perform better in a rule switching paradigm. Unfortunately, this is not a manageable paradigm given our current setup as a low percentage of animals reach criteria. We need to determine a more robust paradigm to test executive function and various alternative methods are currently being evaluated. Unfortunately we were not able to test AVL-3288 in this paradigm within the allotted time because of the aforementioned circumstances. Nevertheless we believe that the weight of the evidence supports a role for the activation of the α7 nAChR in improving cognitive function in general and specifically the nootropic effects of AVL-3288 including effects on executive function helps to mitigate our shortfall in completing this task (Table 2).

Table 2. Effect of AVL-3288 on cognitive function in various preclinical models of learning and memory

Model	Functional Domain	Species	MED* or active doses (mg/kg)	Route	Investi-gators**	Publication
SENSORY GATING DEFICIT	Pre-attention	DBA/2 mouse	0.3 *	i.v	1	Ng et al., PNAS, 104:8059-8064, 2007
RADIAL ARM MAZE	Working & reference memory	S-D rats	0.3 *	i.p	2	Ng et al., PNAS, 104:8059-8064, 2007
NOVEL OBJECT RECOGNITION	Working memory	CD-1 mouse	0.1 *	i.p	2	Unpublished or published with related compounds (Hogenkamp et al., J Med Chem 56:8352-8365, 2013)
NOVEL OBJECT RECOGNITION	Working memory	S-D rats	0.3 - 3	p.o.	3	Nikiforuk et al., European Neuro-psychopharmacology 25: 1300-1313, 2015
5-CSRTT	Attention, impulsivity, procedural memory, executive function	S-D rats	0.3 *	i.p.	2	Unpublished in-house data
MORRIS WATER MAZE	Hippocampal based spatial learning and working memory	S-D rats	0.3	i.p.	4	Atkins et al., submitted for publication
FEAR CONDITIONING	Working memory and recall	S-D rats	0.3	i.p.	4	Atkins et al., submitted for publication
SOCIAL DISCRIMINATION	Working memory	Wistar rats	1	s.c.	5	Thomsen et al., PLOS One, 6:e27014-e27022, 2011.
ATTENTIONAL SET SHIFTING	Executive function	S-D rats	0.3 - 3	i.p.	3	Nikiforuk et al., European Neuro-psychopharmacology 25: 1300-1313, 2015
KETAMINE-INDUCED NOR	schizophrenia-like working memory deficits	S-D rats	0.3, 1.0	i.p.	3	10.1016/j.neuropharm.2015.07.034
KETAMINE-INDUCED ASST	schizophrenia-like executive function deficits	S-D rats	0.3, 1.0	i.p.	3	10.1016/j.neuropharm.2015.07.034
KETAMINE-INDUCED SOCIAL INTERACTION DEFICITS	schizophrenia-like social interaction deficits	S-D rats	0.3, 1.0	i.p.	3	10.1016/j.neuropharm.2015.07.034

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4. Dept. of Neurological Surgery, University of Miami/Coleen Atkins
5. University Hospital, Copenhagen, Denmark/Jens Mikklesen

Task 2q: Pharmacokinetics – $t_{½}$ and C_{max} MED (BTBR strain)

The half life and C_{max} of the two test compounds in BTBR mice are summarized in table 3. We were unable to detect levels of 2-261 at its MED (0.3 mg/kg) in the social interaction paradigm because the limits of quantitation were exceeded. Consequently we used 10 mg/kg i.p. to derive these values. Extrapolation of the C_{max} from the 10 mg/kg dose, assuming dose proportionality, would put the levels at ~0.04 µM whereas the $t_{½}$ should stay the same. The values for AVL-3288 were also based on the MED (3 mg/kg i.p.) in the social interaction paradigm.

Table 3. Pharmacokinetics of 2-261 (10 mg/kg i.p.) and AVL-3288 (3 mg/kg i.p.) as measured in plasma

Compound	$t_{½}$ ± SEM (min)	C_{max} ± SEM (µM)
2-261	82 ± 7	1.3 ± 0.4
AVL-3288	121± 20	0.4 ± 0.1

SEM=standard error of the mean

Task 2r: Sociability - gabazine antagonism (BTBR strain)

The selective $GABA_AR$ antagonist, gabazine, was tested in BTBR mice to determine the effect on 2-261 and AVL-3288 in sociability as measured by the difference in time they spend with a novel object versus a novel mouse (DBA/2) in the sociability paradigm. Gabazine at 3 mg/kg is able to block the improvement in sociability from 2-261 at 0.3 m/kg (Figure 10, left panel). Gabazine at 3 mg/kg is not able to block the improvement in sociability from AVL-3288 at 3 mg/kg (Figure 10, right panel). This suggests that a $GABA_A$ receptor mediated pathway is necessary for the sociability effect of 2-261, but not for AVL-3288.

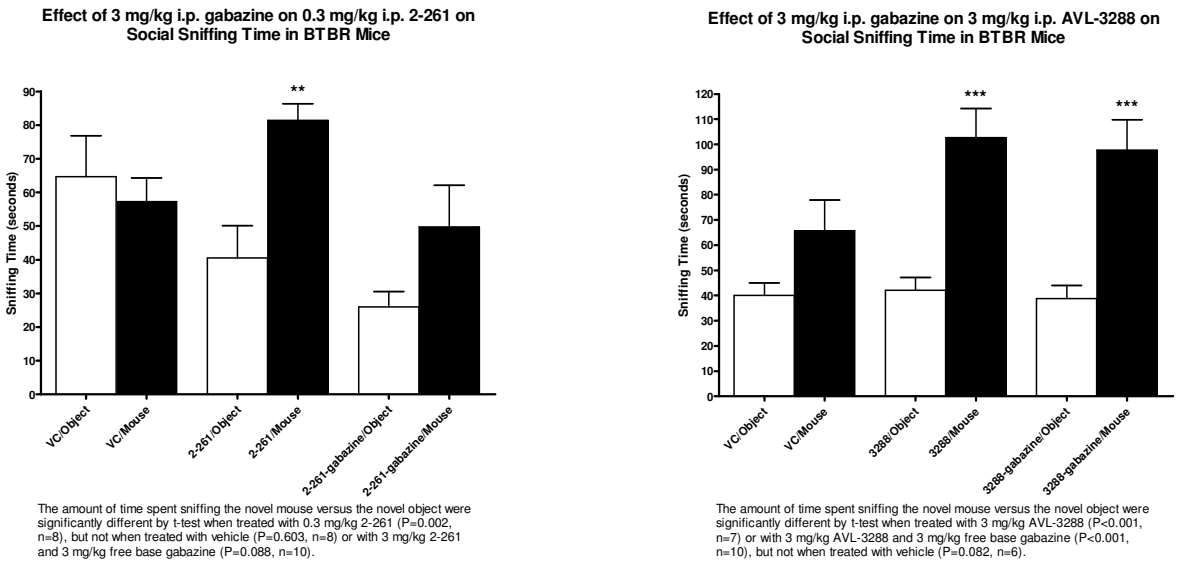

Figure 10: The effect of gabazine (3 mg/kg free base i.p.) on 2-261 (left panel, 0.3 mg/kg i.p.) or AVL-3288 (right panel, 3 mg/kg i.p.) on sociability in BTBR mice. Sociability was recorded as the time in seconds (sec) spent sniffing the novel mouse (filled bars) versus the novel object (open bars) starting 30 minutes after vehicle or drug administration.

Task 2s: Sociability - MLA antagonism (BTBR strain)

The α7 nAChR antagonist, methyllycaconitine (MLA), was used to determine whether the α7 nAChR contributed to the actions of both 2-261 and AVL-3288 on sociability as measured by the difference in time they spend with a novel object versus a novel mouse (DBA/2) in the sociability paradigm. MLA (3 mg/kg) did not antagonize the effect of the $GABA_AR$ PAM 2-261 at 0.3 mg/kg which suggests that the action of 2-261 on sociability is not mediated by the α7 nAChR (Figure 11, left panel). In contrast, the effect of α7 nAChR PAM, AVL-3228, on sociability was antagonized by MLA (Figure 11, right panel). These studies combined with those using gabazine suggest that two different sites of action/pathways may be activated to improve social interaction in BTBR mice. This is a novel finding which indicates the both the α7 nACh and $GABA_A$ receptors can independently improve sociability in BTBR mice.

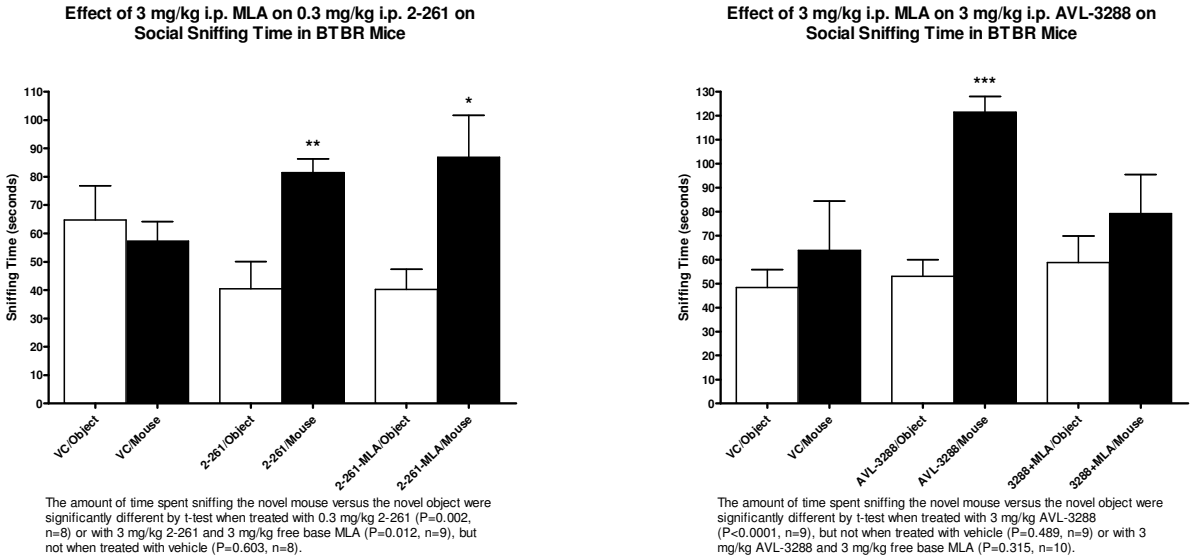

Figure 11: The effect of MLA (3 mg/kg free base i.p.) on 2-261 (left panel, 0.3 mg/kg i.p.) or AVL-3288 (right panel, 3 mg/kg i.p.) on sociability in BTBR mice. Sociability was recorded as the time in seconds (sec) spent sniffing the novel mouse (filled bars) versus the novel object (open bars) starting 30 minutes after vehicle or drug administration.

Task 3a and 3b: Sociability - combination drug testing (BTBR strain)

Non-effective doses of 2-261 (0.1 mg/kg) and AVL-3288 (1 mg/kg) in the sociability paradigm were combined and tested for activity in enhancing social sniffing in BTBR mice. In contrast to their lack of activity alone, the combined administration of the two compounds results in a significant improvement in sociability (Figure 12). These observations support the rational for the use of a dual modulator of both receptors to reduce the dose of drug required to produce a therapeutic effect.

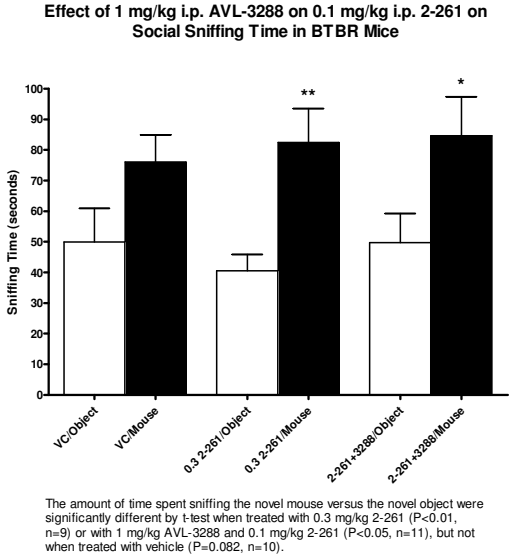

Figure 12: The effect of a combination of the no effect doses of 2-261 (0.1 mg/kg i.p.) and AVL-3288 (1 mg/kg i.p.) compared to the minimum effective dose of 2-261 (0.3 mg/kg i.p.) on sociability in BTBR mice. Behavior was recorded as the time in seconds (sec) spent sniffing the novel mouse (filled bars) versus the novel object (open bars) starting 30 minutes after vehicle or drug administration.

Task 3c: Self-grooming - combination drug testing (BTBR strain)

2-261 is ineffective at any dose in self-grooming, so combination studies in Task 3c were not performed.

Task 3d: 5-CSRTT - combination drug testing (BTBR strain)

Task was not completed; please refer to discussion under tasks 2n-2p

Task 4a and 4b: Sociability - drug testing (BTBR strain)

The effect of vehicle, GRN-529 and the dual α7 nACh and $GABA_A$ receptor PAM, 40327, were tested for their effects on sociability in BTBR mice (Figure 13). The expectation was 40327 would be active because both 2-261 and AVL-3288 were active in this paradigm. The results indicate that indeed 40327 was active as predicted by the activities of 2-261 and AVL-3288. Since the $GABA_A$ receptor PAM 2-261 was also active in this model, the most likely explanation is that the activity of 40327 is mediated by both receptors. The relative contribution of each receptor to the activity of 40327 will be determined by the PK/PD relationship. This will be established in subsequent studies starting with the development of a bioanalytical assay for 40327. The expectation is that levels of 40327 at its MED will be below the threshold for activation of the individual receptors. This prediction is supported by the data that demonstrates the activity of subthreshold doses of 2-261 and AVL-3288 on sociability (see figure 12). These PK/PD studies are beyond scope of the studies covered by the current proposal.

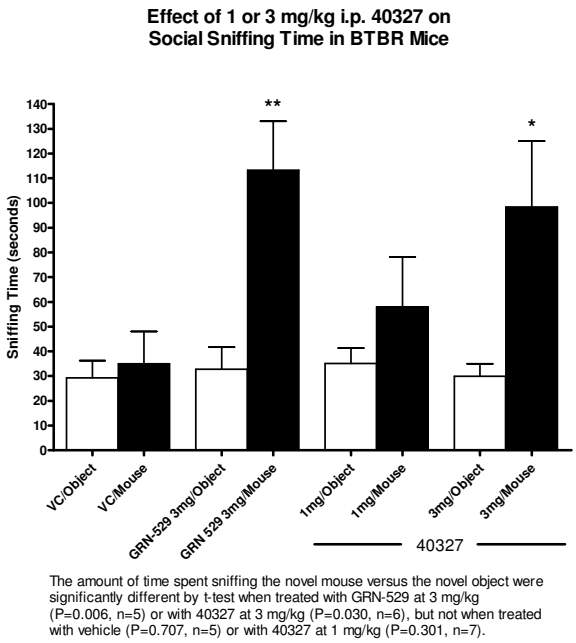

Figure 13: The effect of a 40327 (1.0 & 3.0 mg/kg i.p.) compared to the GRN-529 (3 mg/kg i.p.) on sociability in BTBR mice. Behavior was recorded as the time in seconds (sec) spent sniffing the novel mouse (filled bars) versus the novel object (open bars) starting 30 minutes after vehicle or drug administration.

Task 4c: Self-grooming - drug testing (BTBR strain)

The effect of 40327 versus vehicle and GRN-529 was measured in self-grooming in BTBR mice (Figure 14). The expectation was, despite 2-261's lack of activity in this paradigm, 40327 would be active because of its AVL-3288-like activity at α7 nAChR's. The results indicate that indeed 40327 was as active as GRN-529 in this paradigm. Since the $GABA_A$ receptor PAM 2-261 was inactive in this model, the most likely explanation is that the activity of 40327 is mediated by the α7 nAChR.

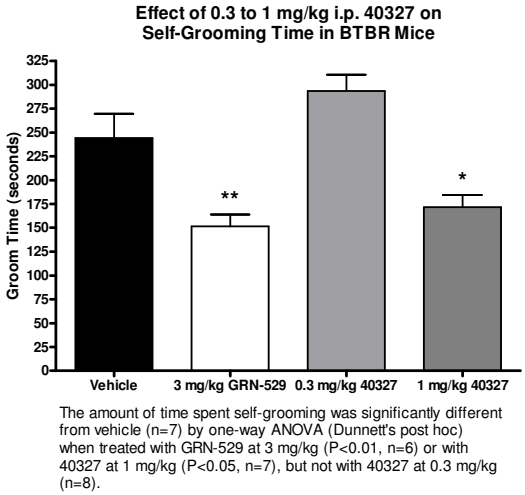

Figure 14: The effect of GRN-529 (3 mg/kg i.p.) or 40327 (0.3 to 1 mg/kg i.p.) on self-grooming behavior in BTBR mice. Self-grooming behavior was measured 30 minutes after vehicle or drug administration and the amount of time spent grooming was recorded during a 10 minute observation period.

Task 4d: 5-CSRTT - combination drug testing (BTBR strain)

Task was not completed; please refer to discussion under tasks 2n-2p

4. KEY RESEARCH ACCOMPLISHMENTS:

- *Established a BTBR mouse colony and demonstrated behavioral phenotypes similar to certain core symptoms observed in ASD thus providing a useful model of the disease with face validity*

- *Demonstrated that a $GABA_A$ receptor subtype selective PAM, 2-261, ameliorates the deficits of reduced social interaction in the BTBR mouse model of ASD thus providing key data in support of the hypothesis that deficits in inhibitory neurotransmission may contribute to a core symptom of ASD*

- *Demonstrated that an α7 nAChR receptor subtype selective PAM, AVL-3288, ameliorates the deficits of reduced social interaction and excessive self-grooming (i.e.,, stereotypy) in the BTBR mouse model of ASD thus providing key data in support of the hypothesis that deficits in α7 nAChR receptor mediated neurotransmission may contribute to these core symptoms of ASD*

- *The activity of AVL-3288 in correcting both social interaction and stereotypy deficits in BTBR mice predicts that the dual α7 nACh and $GABA_A$ receptor PAM, 40327, will also be active in both paradigms. Indeed, 40327 is active with MED's of 1 and 3 mg/kg in the self-grooming and sociability paradigms, respectively*

- *The PAMs that we tested have an obligatory reliance on endogenous levels of neurotransmitter are active in the BTBR model of ASD and thus support the contention that endogenous neurotransmission is sufficient for PAMs to have positive impact on abnormal behavioral activity in BTBR mice*

- *The data generated in the current project combined with the existing data on 2-261 and AVL-3288 provide compelling support for the optimization and development of a <u>dual</u> α7 nACh and $GABA_A$ receptor PAM for the treatment of the core symptoms and associated neurological/medical symptoms (e.g., anxiety, seizures, aggression, hyperactivity, inattention and mood disorders)of ASD*

- *The observation that AVL-3288 is active in both social interaction and stereotypy deficits in BTBR mice provides a strong rationale to test AVL-3288 clinically since the compound is already in phase 1b (NCT01851603) clinical development for cognitive impairment in schizophrenia*

5. CONCLUSION:

Our findings support the hypothesis that the potentiation of inhibitory mechanisms mediated by $GABA_A$ receptor subtypes is a viable strategy to ameliorate one of the core symptoms of ASD, reduced social interaction. Unfortunately the BZs, the canonical $GABA_A$ receptor PAMs, are not ideal drugs because of their side effect profile and abuse potential. In contrast, the $GABA_A$ receptor PAMs represented by 2-261 are devoid of these adverse effects in our preclinical models (Gee et al., 2010; Yoshimura et al., 2014). Thus our data provide the foundation for the first series of compounds that may be developed as highly selective $GABA_A$ receptor subtype PAMs that avoid the problems of the BZs. When combined with our observation that α7 nAChR PAMs correct both deficits of social interaction, stereotypy and their well documented effects in improving cognitive function, the design of a dual modulator of both LGICs becomes desirable and feasible. Collectively the data provide the proof-of-concept originally proposed in our grant that such a dual-action compound will allow full coverage of the core symptoms as well as the neurological/medical symptoms of ASD. The prototype of such a dual-action modulator is 40327 which will provide the basis of a lead optimization program for the first drug purposefully designed to selectively and <u>simultaneously potentiate</u> two complementary subsystems in the brain for the treatment of ASD.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Publications: Nothing to report

Abstracts: Nothing to report

Presentations: Yoshimura RF, Tran MB, Hogenkamp DJ, Egusquiza R, Gee TK, Gee KW (October 2014). *Effect of $GABA_AR$ subtype-selective positive allosteric modulators in the BTBR mouse model of autism*. Poster presented at Society for Neuroscience Annual Meeting 2014, Washington, DC.

7. PERSONNEL ON GRANT

a. Timothy Johnstone

b. Derk Hogenkamp

c. Minhtam Tran

d. Ryan Yoshimura

8. INVENTIONS, PATENTS AND LICENSES: Nothing to report.

9. REPORTABLE OUTCOMES: Nothing to report.

10. OTHER ACHIEVEMENTS: Nothing to report.

11. REFERENCES:

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12. APPENDICES: None